Title: Brucellosis endocarditis with methicillin-resistant *Staphylococcus aureus* (MRSA) superinfection – Case report from the Country of Georgia

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ABSTRACT

Background

A case of brucellosis endocarditis, a rare complication of brucellosis, was detected as part of an undifferentiated febrile illness surveillance study conducted in Georgia. The case was further superinfected with methicillin-resistant *Staphylococcus aureus* (MRSA) during the hospital stay. To our knowledge, this is one of only a few reports of brucellosis endocarditis cases with MRSA superinfection.

Case presentation

A 56-year-old housewife, a resident of the southern part of Georgia, presented to the hospital for undifferentiated febrile illness of one month's duration and was enrolled in the febrile illness surveillance study. Fever, shaking chills, sweating, arthralgia and myalgia, nausea, fatigue, and malaise were among her chief complaints. Her condition did not resolve on empiric antibiotic therapy. Severe mitral valve stenosis with 8-mm and 10-mm vegetations on the posterior and anterior leaflets, respectively, were reported by echocardiography. Brucellosis serology was positive by both enzyme-linked immunosorbent assay and agglutination assay. *Brucella abortus* was isolated from blood culture, and removed cardiac valve tissue was positive for the same pathogen by molecular assays. After developing ischemic stroke and splenic infarction, the patient underwent valve replacement surgery. One of her blood cultures taken after hospital admission was positive for MRSA. Amikacin (15 mg/kg daily for 10 days) and a prolonged course of doxycycline (100 mg bid) was prescribed after establishment of the brucellosis diagnosis. Vancomycin (1 g, administered intravenously q12h) was added for MRSA coverage, and rifampin was initiated to augment brucellosis treatment.

Summary

The case of brucellosis endocarditis with MRSA superinfection described here is anecdotal evidence of the rare etiologic structure of infective endocarditis.

Key words: brucellosis, MRSA, endocarditis

SHORT REPORT

Background

Brucellosis is a chronic febrile illness, characterized by a granulomatous inflammatory reaction affecting almost all organs and systems and requiring prolonged treatment with a combination of antibiotics [1]. Fever, constitutional symptoms, hepatosplenomegaly, lymphadenopathy, arthritis, and epididymoorchitis are common manifestations of the disease. Rarely, involvement of the respiratory and/or central nervous systems is observed as well [1]. Endocarditis is one of the rare but potentially fatal complications of brucellosis. Although endocarditis is quite rare in non-endemic areas, it may reach up to 11% in endemic areas [2]. The aortic valve is affected primarily, but the mitral valve can be involved as well [1, 3]. A combination of long-term antibiotic therapy and surgical valve replacement improves the final outcome [2, 4].

Brucellosis is an endemic zoonotic disease in Georgia with the first laboratory-confirmed cases described in 1923 [5]. Between 150 and 200 cases are registered annually, and laboratory diagnosis is based primarily on qualitative slide (Huddelson) and quantitative tube (Right) agglutination assays [5, 6]. The Right agglutination test cut-off is set at 1:200 in Georgia.

An active surveillance study for undifferentiated febrile illness cases was established in June 2013 to describe the frequency of the selected infectious agents in the structure of undifferentiated febrile illnesses in the country of Georgia. Patients ≥ 4 years old without an established diagnosis were eligible for enrollment if they had: (1) a temperature of ≥ 38°C for ≥ 48 hours and/or (2) hemorrhagic fever syndrome. Standard questionnaires were completed and blood samples were collected as part of this surveillance study. Blood culture was conducted with a focus on the identification of common bacteria as well as *Brucella* spp. Isolated *Brucella* spp. culture was subjected to initial biochemical testing followed by DNA extraction. Isolates were first analysed by real-time polymerase chain reaction (PCR) using Brucella Target 1 (BioFire Diagnostics, USA). PCR-positive *Brucella* spp. samples were then subjected to a modified Bruce-ladder PCR assay (an adaptation by Su and Nikolich of an assay assembled and validated at Walter Reed Army Institute of Research, USA, identifying isolate as *B. abortus* [7]). Additionally, the enzyme-linked immunosorbent assay (ELISA) was conducted to detect IgM antibodies against *Leptospira* (PanBio, Australia) and *Coxiella burnetii* (PanBio, Australia). Both IgM and IgG antibodies were detected against *Brucella* (IBL-International, Germany), Crimean–Congo hemorrhagic fever virus (Vector-Best, Russia), hantavirus (IBL-International, USA), and *Rickettsia* spp. (Naval Medical Research Center in-house ELISA, USA), including spotted fever group rickettsiae, scrub typhus group orientiae, and typhus group rickettsiae.

Here we report a case of brucellosis endocarditis, a rare complication of brucellosis identified as part of this surveillance effort.

Case presentation

A 56-year-old housewife, a resident of Akhaltsikhe, a city in the southern part of Georgia, presented to the V. Bochorishvili Sepsis Center in July 2014 for undifferentiated febrile illness of one month's duration and was enrolled in the febrile illness surveillance study. Her diagnosis remained unclear despite thorough investigations performed by local infectious disease and tuberculosis specialists. Her condition did not resolve on empiric antibiotic therapy consisting of Penicillin-G, Ceftriaxone, and levofloxacin.

The admission diagnosis was infective endocarditis (IE). Her major complaints were fever, shaking chills, sweating, arthralgia and myalgia, nausea, fatigue and malaise. Pallor, jaundice, cervical lymphadenopathy, heart murmur, and hepatosplenomegaly were observed on physical examination. Travel history within or outside Georgia during the month prior to disease onset was negative. The presence of tick or flea bites; engagement in agricultural activities; and exposure to household pets (a cat and a dog), farm animals (goats and cattle), and rodents during the same period was reported. Extensive laboratory and radiologic investigations were performed. Echocardiography revealed severe mitral valve stenosis with 8-mm and 10-mm vegetations on the posterior and anterior leaflets, respectively. *B. abortus* was isolated from blood cultures. Serum was positive for brucellosis IgM and IgG (performed by ELISA). Brucellosis agglutination assay detected a titer of 1:1600. All other tests conducted as part of the febrile illness surveillance study were negative.

The patient was prescribed amikacin (15 mg/kg daily for 10 days) and a prolonged course of doxycycline (100 mg bid). Despite adequate antibiotics, the patient's condition deteriorated. By the end of July, thromboembolic complications developed with ischemic stroke and splenic infarction. The patient was admitted to the intensive care unit (ICU). Blood culture collected in the ICU yielded methicillin-resistant *Staphylococcus aureus* (MRSA). Vancomycin (1 g

administered intravenously q12h) was added for MRSA coverage, and rifampin was initiated to augment brucellosis treatment. Doxycycline was continued. Rifampin was discontinued because of side effects later. In early September, 3 months after disease onset, surgical mitral valve replacement was conducted at the Kipshidze Central Clinical Hospital in Tbilisi. The removed mitral valve culture was negative for MRSA and *Brucella* spp., but PCR identified *B. abortus* genome in the sample (Figure 1). Moreover, genotypes of *B. abortus* in blood and mitral valve appeared to be identical when subjected to Multiple Locus Variable Tandem Repeat Analysis (MLVA – 15) targeting 15 markers with subspecies discriminatory capabilities (Su and Nikolich adaptation of assembled and validated in-house at WRAIR, USA [8]). Since the patient did not show up for the follow-up visit final outcome data is not available.

Conclusions

To our knowledge, this is the first documented case for decades of brucellosis endocarditis—with microbiological, molecular, and serological evidence—in Georgia [5]. A combination of early surgical intervention and brucellosis-specific treatment is required for the best prognosis [2, 4]. Due to delayed surgical intervention, the reported case developed splenic and neurologic embolism, which further deteriorated her condition and led to her admission to the ICU. Septic emboli are frequent life-threatening complications affecting one-third of patients with IE [9]. Brucellosis is not a common cause of IE, with a global incidence of 1% [10]. Despite its rarity, brucellosis should be suspected in the differential diagnoses of the patient who presents with prolonged undifferentiated fever from certain parts of Georgia. MRSA bloodstream infection is a common nosocomial infection associated with an ICU stay [11]. In the case reported here, the

isolation of MRSA in the bloodstream later in the course of the disease was most likely a superinfection acquired in the hospital during the patient's ICU stay because MRSA was not identified during several weeks prior to hospitalization, and MRSA was not revealed with culturing and PCR testing of the excised valve.

This case report underlines the importance of clinical vigilance considering endemic diseases during differential diagnosis of febrile patients. Additionally, the superinfection incident reveals diagnostic and patient care challenges intrinsic to real-life clinical practice, where cases do not match textbook presentations.

List of abbreviations

MRSA, methicillin-resistant *Staphylococcus aureus*; IE, infective endocarditis; ICU, intensive care unit; bid, twice a day; q12h, every 12 hours.

Ethics approval and consent

The study protocol was reviewed and approved by the institutional review boards of the National Center for Disease Control and Public Health of Georgia and Walter Reed Army Institute of Research. Informed consent was obtained from each participant prior to initiation of any study-related activity.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Tinatin Kuchuloria, Paata Imnadze, Tamar Akhvlediani, Maiko Chokheli, Robert G Rivard, George Kandelaki, Christian T Bautista, and Nino Trapaidze were involved in study design development. Marina Mamulashvili was involved in screening eligible patients. Marine Kanashvili, Manana Makhviladze, and Teona Mikautadze were involved in consenting and data collection. Marine Ramishvili, Lile Malania, Nazibrola Chitadze, Marina Chubinidze, Ekaterine Zhgenti and Ketevan Sidamonidze provided laboratory support. All authors have actively participated in the development of the manuscript.

Authors' information

NA

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Endnotes

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FIGURE 1

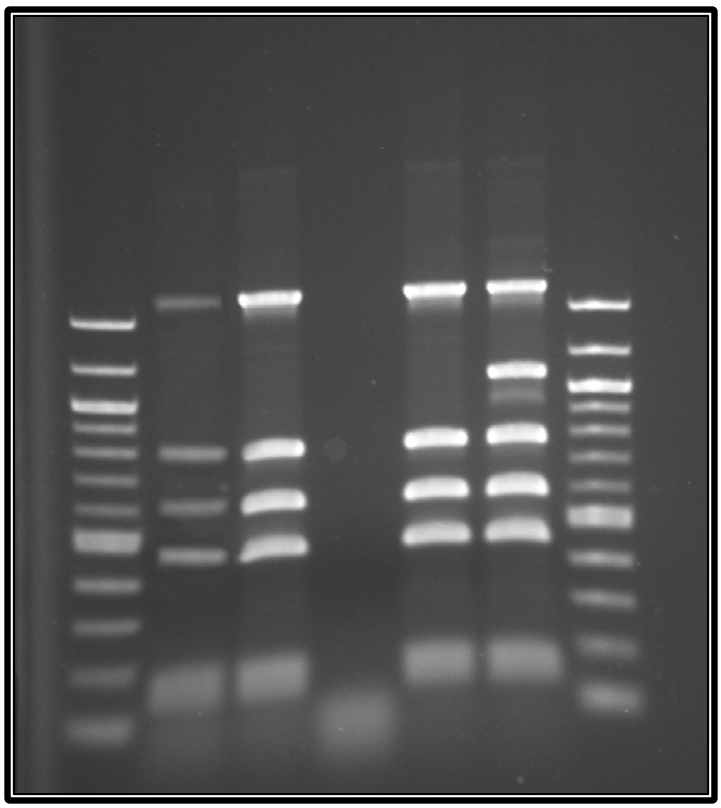

Figure 1. From left to right. Lane 1: 100-bp ladder. Lane 2: DNA isolated from surgically removed mitral valve, subject #173. Lane 3: DNA isolated from *B. abortus* isolate obtained from the blood culture of subject #173. Lane 4: No DNA. Lane 5: *B. abortus* DNA (positive control). Lane 6: *B. melitensis* DNA (positive control). Lane 7: 100-bp ladder.